A Synopsis Entitled

“PRODUCTION AND OPTIMIZATION OF PECTINASES PRODUCED BY SOME FUNGI FROM FRUIT PEELS OF NANDED DISTRICT”

Submitted to

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Abstract of proposed work plan/ problem:

Production and Optimization of pectinases produced by some fungi from Fruit peels of Nanded district.

1. Introduction

Pectin is a polymeric material having carbohydrate group esterifies with methanol. It is an important component of plant cell wall. It is present in highest concentration in the middle lamella, where it acts as a cementing substance between adjacent cells. Plant pathogens attack target cells by producing number of cell degrading enzyme which facilitate the entry and expansion of pathogen in the host tissue.1 Pectinolytic enzymes are of significant importance in the current biotechnological era with their all embracing applications in fruit extraction and its clarification. In addition, are involved in degumming of plant fibers, vegetable oil extraction, tea and coffee processing and alcoholic beverages etc. They have a share of 25% in the global sales of food enzymes . Carbon sources especially of agrarian source are more suitable because they are cost effective, renewable and available in large quantities.

Pectinase is a group of enzymes that is involved in the breakdown of pectin. It also breaks glycoscidic linkages by splitting polygalacturonic acid into monogalacturonic acid (Sethi, et al., 2016; Abdullah, et al., 2018). Pectinases have crucial roles in food industries. These enzymes are useful for fruit juice extraction and wine clarification; tea, cocoa, and coffee concentration and fermentation; vegetable oil extraction; preparation of jam and jellies; and pickling (Barman, et al., 2015; Kubra, et al., 2018 ; Sudeep, et al., 2020). Furthermore, these enzymes are used in paper and pulp industries, bleaching of paper, bioscouring of cotton, retting and degumming of plant fibers, oil extraction, wastewater treatment, poultry feed additives, protoplast fusion technology, and bioenergy production (Oumer, 2017; Kubra, et al., 2018).Pectinases are enzymes involved in the hydrolysis of pectin. Pectin, the constituent of primary cell wall of terrestrial plants, was first isolated and described by Henri Braconnot in 1825. It is a high molecular weight heteropolysaccharide rich in arabinose, galactose, and galacturonic acid with a rhamnogalacturonan backbone. It forms a jelly-like substance in the cell wall and exists as calcium and magnesium pectate. In addition to the plant cell wall, it is also present in fruits in very high concentrations. Pectin also has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of drugs, proteins and cells.

During ripening, pectinase breaks pectin into simple sugars, which results in the sweetening and loosening of fruits. There are different types of pectin like protopectin, pectinic acid, gelatinous acid, and pectic acid. Pectinases is a broad term that covers three different enzymes: Pectinesterases (PE) act upon ester bonds in pectin breaking them into simpler forms, Polygalacturonases (PG) target the bonds between the galacturonosyl residues, and Pectatelyases (PL) target the alpha glycosidic bond. Pectinases are classified as exo and endopolygalacturonases depending on the position of bond cleavage.

Many studies have been conducted on the production, isolation and characterization of pectinases from various microorganisms, mainly enzymes specific to pectin but a few works have been published about pectin extracted from Agrowastes and production of pectinolyticenzymes.Therefore the problem is undertaken for study.

Review of Literature and Development in the subject (Previous work done in the relevant area):

Fungi are used for producing low-cost pectinase enzymes for industrial purposes. Media and time are the most important factors for increasing production rate. Pectinase is a heterogeneous group of enzymes that hydrolyze pectin 9 .They are widely used in the food industry to improve the cloud stability of fruit and vegetable nectars,for production and clarification of fruit juices, and for haze removal from wines (Cavalitto et al., 1996). Furthermore, phytopathologic studies have reported that fungal endo-polygalacturonase (endoPGase) which is a major kind of pectinase has been shown to activate plant defense responses, including phytoalexin accumulation, lignification, synthesis of proteinase inhibitors, and necrosis (Cervone et al., 1989). Further research has confirmed that endoPGase can degrade the plant cell wall releasing pectic oligomers which can stimulate a wide array of plant defence responses (Boudart et al., 1998). With the increasing application of pectinases, decreasing its production cost has become one of the most important targets. For this purpose, selection of carbon source and nitrogen source with low value is a practical consideration. Previous studies reported that many waste products from the agricultural industry containing pectin, such as sugar beet pulp (SBP), citrus pulp pellets, apple pomace, pulp, lemon pulp and other related materials have been used as carbon source for induction of pectinase by many microorganisms (Said et al., 1991).

The pectic substance is mainly found in the middle lamella and primary plant cell wall. Pectinases can be obtained from various strains of bacteria and filamentous fungi. *A. niger* is used to obtain pectin from waste material with flask fermentation and bioreactor cultivation. Pectin is the most important factor that affects the amount of pectinases produced, and many other factors are also responsible, and they are measured with Pareto chart of standard effects. Bioreactor cultivation should be done with various control methods. This method of obtaining pectinases is highly useful for industries

Objectives of Research/ Proposed Hypothesis:

The specific objectives of this study are

To collect fruit samples from Nanded district

To extract pectin from fruit peels

To isolate pectin degrading fungi from infected fruits

To produce pectinases by using crude pectins or pectin peptone medium

To measure pectinase enzyme activity

To optimize medium conditions for enzyme production

To find out best source of pectin and pectinase producing fungi

Methodology to be adopted:

Extraction Process

Pectin extraction will be conducted according to Khule et al. (2012), with slight modifications.

Characterization of Pectin

The extracted pectin will be characterized in terms of moisture content, ash content, methoxyl content, degree of acetylation, intrinsic viscosity and molecular weight. The dried pectin samples obtained from orange peels will be subjected to the following quantitative and qualitative tests in order to characterize them.

Qualitative Test for Pectin

Test for pectin was carried out according to USP (2007) standard method.

Isolation and identification of pectinolytic micro fungi from infected fruits

The filamentous fungi will be isolated from decaying fruit agro waste matter using a basal medium containing commercial pectin as the sole carbon source.

Pure cultures will be obtained by repeated sub- culturing on PDA plates and maintained at $40^{o}C$ on PDA slants.

The isolate fungal colonies were identified based on their colonial and morphological characters.

Screening of fungal isolates for pectinolytic activity

Assay of crude enzyme:

The activity of crude enzymes will be determined by measuring the release of reducing sugar using dinitrosalicylic acid reagent DNS assay (Miller., 1959). The standard graph of glucose will be plotted . From the standard graph the activity of crude enzyme will be calculated

Characterization of crude enzyme:

The characterization of crude enzyme sample was done. The effect of temperature, pH, time course, substrate concentration, enzyme concentration and metal ions was studied

Enzyme production

The fungal mother culture was used to produce pectinase enzyme using liquid medium containing dextrose 2%, citrus pectin 1% at pH 6. Fermentation was carried out in a test tube containing 5 mL of liquid medium with 100µl mother culture of R. oryzae and incubated at 30 °C for 7 days (Angayarkanni et al., 2002). The biomass was separated by filtration. The supernatant was used to evaluate the polygalacturonase enzyme activity.

Sample harvesting

After specific interval of incubation time, the biomass from the experimental test tubes will be separated by filtering through Whatman filter paper no.1. The filtrate will be centrifuged at 5,000 rpm for 15 minutes in the centrifuge (cat. no.: CF, 405, 5000 rpm. Gallenkamp, England) to remove the spores and mycelia of the organism. The supernatant will be carefully collected and will be stored at refrigerated temperature in sterilized test tubes.

Enzyme activity

.Polygalacturonase enzyme activity will be measured by using DNS method (Miller, 1959; Carmona et al., 1998). Enzyme activity will be expressed as U.mL-1 .

Unit of activity

According to the International Union of Biochemistry, one international unit of pectinase (1 U) corresponds to the amount of enzyme required to release 1 micromole of reducing substance (GA) in 1 minute. Enzyme activity will be expressed as U.mL

Calculation of enzyme activity

The enzyme activity will be calculated by the following formula

$$\text{Activity} = \frac{\text{Dilution} \times \text{Absorbance}}{\text{Time of incubation (min)}} \times \text{Standard Factor}$$

$$\text{Standard factor} = \frac{\text{Concentration of standard solution (µmole/mL)}}{\text{Absorbance at 550 nm}}$$

Optimization of Pectinase production

Medium carbon source (dextrose) will be replaced with pectin Pectin will be used separately at different concentrations (0.5, 0.75 and 1.0%) in the medium. The fermentation will be carried out for a period of 120 hours keeping other components

- Effect of pH value:
- Effect of incubation temperature:
- Effect of incubation periods:
- Effect of different carbon sources:
- Effect of different nitrogen sources:
- Effect of different mineral salts:

.Importance of study/ Society application:

Pectinase enzyme has adjustable catalytic activity, and it is non-toxic to humans. It can be easily produced by a large number of techniques at a cheap rate with good quality of yield.

Microorganisms and plant material are the rich sources of pectinase production . Applications of Pectinase:

Pectinase enzyme is a most significant enzyme in various industries. It is mainly obtained from microorganisms as they produce a very good yield. It is used in industries like the textile industry, paper industries and vegetables, and fruits processing industries, paper and pulp industries, wine industries, animal feed industries, tea and coffee industries etc. There are many more industries that are going to be dependent on pectinase enzyme production for various uses in the future. It is used in industries for avoiding chemicals that may or may not produce some harmful effects on the environment, humans, flora and fauna .

The production of pectinases is eco-friendly because in this, only agro and food wastes can be used. Pectinases are used in different industrial sectors like fruit juice extraction, coffee, and tea fermentation, textile industry, fruit juice industry, etc. As pectinase is used widely in the industry so whenever the degradation of pectin is needed, it is important to undertake research in the screening of microorganisms for pectinases and investigate the optimal conditions for the production of microbial pectinases.

International status

Enzyme is an essential tool in juice processes, both in terms of quality improvement and cost saving (Ramadan and Moersel, 2007). Now a day's production of fruit and vegetable juice is almost unthinkable without the use of enzymes (Baumann, 1981). The degradation of plant cell walls by exogenous enzymatic treatment results in easier release of the components contained in cells (Janser, 1997). The utilization of microbial enzymes has found broad technological application in different industrial processes. Among the various enzymes commercialized many are products of fermentation of filamentous fungi (Piccolivalle et al., 2001). Pectinases are the groups of enzymes, which cause degradation of pectin that, are chain molecules with a rhamnogalacturonan backbone, associated with other polymers and carbohydrates. These pectinases have wide applications in fruit juice industry and wine industry. In fruit juice industry, it is used for clarification, where reduction in viscosity is caused which ultimately leads to formation of clear juice. Pectinases can be produced by both submerged and solid state fermentation (SSF). Submerged fermentation is cultivation of microorganisms on liquid broth. It requires high volumes of water, continuous agitation and generates lot of effluents. Solid state fermentation incorporates microbial growth and product formation on or within particles of a solid substrate (Mudgett, 1986) under aerobic conditions, in the absence or near absence of free water, and does not generally require aseptic conditions for enzyme production. Many filamentous fungi like *Aspergillusniger, Aspergillusawamori, Penicilliumrestrictum, Trichodermaviride, Mucorpiriformis,* and *Yarrowialipolytica*are used in both submerged as well as solid state fermentation for production of various industrially important products such as citric acid and ethanol. Fungi can produce both intracellular as well as extracellular enzymes. All fungi are hetrotrophic, and rely on carbon compounds synthesized by other living organisms. Small molecules like mono disaccharides, fatty acids and amino acids can easily pass through but for breaking down of larger complex compounds like pectin, fungi secrete extra cellular enzymes. It is well known that as compared to intracellular enzymes, the extra cellular enzymes are easier to

be extracted. Intracellular enzymes require more time and costly chemicals for extraction (Hankin and Anagnostakis, 1975).

National Status

Pectins are high molecular weight acid polysaccharide primarily made up of α (1→4) linked D-galacturonic acid residues. They occur as structural polysaccharides in the middle lamella and primary cell walls of young plant cells (Kashyap et al. 2001), where they contribute to the firmness and structure of plant tissues (Sathyanarayana and Panda. 2003). Pectinases are a group of enzymes that catalyze the breakdown of pectins. They are classified into protopectinases, esterases and depolymerases based on their preferred substrate, the degradation mechanism and the type of cleavage (Kashyap et al. 2001). The main sources for the pectinolytic enzymes are yeast, bacteria, and a large variety of filamentous fungi for which the most relevant ones are Aspergillus strains which are generally regarded as safe (GRAS) in food industry (Sumantha et al. 2005) .

Pectinase (EC.3.1.1.11) is a group of enzymes that is involved in the breakdown of pectin. It also breaks glycosidic linkages by splitting polygalacturonic acid into monogalacturonic acid (Sethi et al. 2016). It is a type of polysaccharide made of esterified D-galacturonic acid found in α1-4 chains of middle lamella and primary cell wall of vegetables and fruits. When the ripening process takes place pectinase enzyme breaks down pectin, fruit becomes softer because the cells get separated from each other due to breakage of the middle lamella (Anisa et al. 2013). Global food enzymes sales of pectinases account for 25% (Sethi et al. 2016). Pectinase has a wide range of applications and is a very important enzyme in food industry such as in fruit juice clarification, in the processing of fruits and vegetables.

Significance of the study

The fruit peel wastes resulting from the fruit juice processing industry are normally discarded, which are highly perishable and seasonal; it is a problem to the processing industries and pollution monitoring agencies. Thus, these waste materials may create environmental problems, particularly water pollution, since the presence of biomaterials in fruit waste peel such as peel oil, pectin, as well as sugar can stimulate aerobic bacteria to decompose the biodegradable organic matters into products such as carbon dioxide, nitrates, sulfates and phosphates in water. Suitable methods have to be adopted to utilize them for the conversion of the problem into an asset

This study will contribute to a significant improvement from traditional methods to technological manufacturing of the pectin by experimental work.

This work will show the possibility of getting the jelly pectin and this can be used in pharmaceutical preparations, Anti-cancer action, Food applications industries to improve the performance quality of the product. The possibility of getting the appropriate operating conditions for extraction of pectin.

6. Proposed work Plan/ Formulation and Structure of Study:

Reference work will be done through Journals available by visiting reputed libraries

Collection of fruit samples from different fields of Nanded district

Extraction ofpectins from peels of fruits like Citrus, Sweet lime,Lemon,Banana and pomegranate Characterization of Pectin

Isolation and identification of Pectinolytic fungi from infected fruits by using different media

Screening of fungal isolates for pectinolytic activity

Enzyme production by using crude pectin isolated from fruit peels and pectin peptone medium

Enzyme activity will be carried out spectrophotometrically and Viscosity loss

Optimization of Pectinase production

Ph.D.Thesis will submitted in third year

Year-wise Plan of work and targets to be achieved

First Year

Reference work

Collection of fruit samples

Extraction Process

Characterization of Pectin

Second Year

Isolation and identification of Pectinolytic fungi from infected fruits

Screening of fungal isolates for pectinolytic activity

Enzyme production

Enzyme activity

Third Year

Optimization of Pectinase production

Thesis writing

Submission of Pre Ph. D. Synopsis

Submission of thesis

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